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14. ABSTRACT: We have recently found that CDK5 is active in prostate cancer cell lines and in almost all human metastatic prostate cancers, and inhibition of CDK5 activity resulted in reduction of spontaneous metastases by 79%. In this project, we intend to develop CDK5 as a novel therapeutic target. Therefore, we proposed to characterize a series of small molecule CDK5 inhibitors for specificity in cell culture, and for their effect on xenograft models of prostate cancer. We also proposed to examine the role of CDK5 activity in growth of prostate cancer metastatic to bone, using PC3 based bioluminescent cell clones, and to explore the potential for CDK5 inhibition to sensitize prostate cancer cells to chemotherapy. In the current reporting period, we have found that a small molecule derivative of hymenialdisine is a selective inhibitor CDK5, blocking cell motility without an effect on cell growth. This compound may be useful in limiting metastases in prostate cancer. We have also developed the bioluminescent clones of PC3 cells necessary for our proposed examination of the effect of CDK5 inhibition on prostate cancer cell growth in the bone microenvironment.

15. SUBJECT TERMS
cell motility, metastasis, CDK5, CDK5 inhibitor, molecular imaging

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INTRODUCTION: We have recently found that CDK5 is active prostate cancer cell lines and in almost all human metastatic prostate cancers, and inhibition of CDK5 activity resulted in reduction of spontaneous metastases by 79%. This suggests that CDK5 is a novel potential therapeutic target to limit prostate cancer metastasis. Based on our finding that CDK5 activity is present in prostate cancer and is important for metastasis, we intend to develop CDK5 as a novel therapeutic target. We hypothesized that 1) pharmacological inhibitors of CDK5 can limit or block metastasis, and 2) in established skeletal metastases, inhibition of CDK5 may inhibit tumor growth and sensitize tumor cells to other therapies. Therefore, we proposed to characterize a series of small molecule CDK5 inhibitors for specificity in cell culture, and for their effect on xenograft models of prostate cancer. We also proposed to examine the role of CDK5 activity in growth of prostate cancer metastatic to bone, using PC3 based bioluminescent cell clones, and to explore the potential for CDK5 inhibition to sensitize prostate cancer cells to chemotherapy.

BODY: Note: this report cover the period January 1, 2006- December 31, 2006. In several places in the body of this report, I refer to research done subsequent to this reporting period. In these instances, research done subsequent to the reporting period is *in italics.*

1. Cell culture evaluation of hymenialdisine derivatives as CDK5 inhibitors (months 1-8). We have tested the hymenialdesine compounds in vitro, for their effect on cell motility and cell proliferation, using DU145 cells. Initially we chose to use each of the compounds at a concentration of 2 uM. MTT was used to assay cell proliferation, and a wound healing assay (Strock et al, 2006; ref 1) was used to assay cell motility. At this concentration, compound 28p (see Wan et al, 2004; ref 2) appears to be specific for CDK5, since it had no effect on cell proliferation, but blocked cell motility (Fig.1). The other compounds were effective in blocking cell motility, but also partially inhibited cell proliferation. At 200 nM, compound 28p was not effective for inhibition of cell motility. Thus, the potential for development of compound 28p depends on whether we can maintain these concentrations in vivo (tasks 2 and 4).
2. In vivo toxicity studies of hymenialdisine derivatives (months 9-11). Not done
3. Identification of protein indicators of CDK5 activity (months 5-16). Our initial examination of the phosphorylation of potential CDK5 substrates, based on substrates identified in neuronal cells, has not yet been successful. The candidate proteins we have examined are listed in Table 1. Except for presenilin-1, which was not detected, all candidate proteins were detected, but no change in their phosphorylation was seen.

We are currently examining the phosphorylation pattern of a promising potential indicator, DARPP-32, under conditions of CDK5 activity or inhibition. (*Note: In experiments performed after the close of the first year of this grant cycle, we preliminarily have identified reduced phosphorylation tyrosine 705 of STAT3 as a marker of inhibition of CDK5. This is being pursued, and will be presented in subsequent progress reports.)* In addition, we are looking at the gene expression profile of AT6.3 cells, expressing dominant negative CDK5 or vector-only control (Strock et al, 2006), to try to identify a secreted marker that will indicate CDK5 activity. Such a protein could potentially be a convenient clinical marker for CDK5 inhibition. (*Note: In experiments performed after the close of the first year of this grant cycle, we preliminarily have identified increased expression of LECT2, a secreted protein, as a marker of inhibition of CDK5. This increased expression was initially detected on an Affymetrix cDNA microarray with p <0.01, and has since been confirmed by real-time qRT-PCR. The induction of expression of LECT2 in AT6.3 cells with dominant negative CDK5 is 10-16 fold. This is being pursued, and will be presented in subsequent progress reports.)*

4. Time course studies of CDK5 inhibition in vivo (months 12-15). Not done
5. Evaluation of effect of hymenialdisine derivatives on spontaneous metastasis (months 17-24). Not done
6. Development and characterization of AT6.3luc vector-only, AT6.3luc dnCDK5, PC3luc vector-only, PC3luc dnCDK5 cell clones (months 5-12). We had originally proposed to develop both AT6.3 and PC3 cells, expressing dominant negative CDK5 or vector-only control, with luciferase markers for bioluminescent monitoring in vivo. However, we realized that the AT6.3 cells already have a GFP marker, and can be imaged in vivo by fluorescence. Therefore, we have developed PC3luc (PC3 cells constitutively expressing a lentiviral luciferase construct) clones expressing dominant negative CDK5 or vector-only control. This is now completed, and both the AT6.3 cells and PC3luc cells are being tested in vivo, in the bone microenvironment, as described below.
7. Evaluation of the effect of CDK5 inhibition on skeletal growth of prostate cancer cells (months 13-24). We initially attempted to examine the growth of the AT6.3 cells expressing dominant negative CDK5 or vector-only control, in the bone microenvironment. The cells were injected intratibially, and monitored by GFP fluorescence. Unfortunately, unlike PC3 cells, the AT6.3 cells did not grow in the tibia. One may speculate that this failure to grow in bone may be due to the original selection of these cells from lung metastases. Therefore, we developed PC3luc cells

expressing dominant negative CDK5 or vector-only control. In initial experiments, both the vector-only and the CDK5dn cells did not grow in the tibia (Fig.2). As we showed in our original grant application, this has not been our experience (or that of other researchers; see, e.g., Jenkins et al, Clin. Exp. Metastasis 20:733, 2003; ref. 3) with PC3 cells in the past, and we suspect that we may have, for unknown reasons, selected nontumorigenic clones of PC3 cells during our experiments. We are now testing these cells for their ability to form subcutaneous tumors in nude mice. *At the same time, we have constructed new PC3 cells, infected with a lentivirus expressing a CDK5 shRNA, to block CDK5 expression, or a negative lentiviral control. We are characterizing these cells for their ability to migrate and invade. If these experiments indicate that the cells with the CDK5 shRNA are impaired in their ability to migrate (preliminary experiments indicate that this is the case), we will then examine their ability to form subcutaneous tumors and intratibial tumors in nude mice.*

8. Evaluation of the effect of CDK5 inhibition on sensitivity of prostate cancer cells to chemotherapy (months 25-36). Not done

KEY RESEARCH ACCOMPLISHMENTS:

- Preliminary identification of a selective CDK5 inhibitor
- Development of PC3luc cells expressing dnCDK5

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include: PC3luc dnCDK5 and PC3luc vector only cells; available from the investigators for collaborative projects.

CONCLUSION: We have shown that CDK5 activity is required for metastasis in prostate cancer cells. The identification of a selective CDK5 inhibitor will allow us to explore the potential of CDK5 inhibition by small molecule therapeutics to limit metastasis. In addition, the development of the PC3luc dnCDK5 cells will allow us to test our hypothesis that inhibition of CDK5 will inhibit the ability of prostate cancer cells to grow in the bone microenvironment, the metastatic site that is most lethal in this disease.

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SUPPORTING DATA:

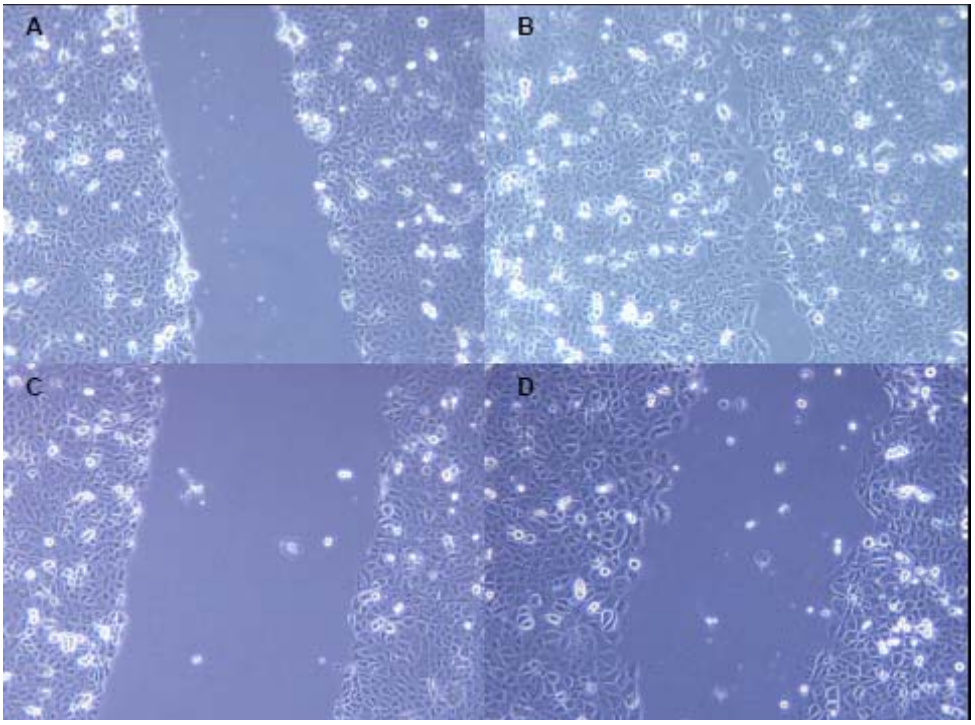

Figure 1. The effect of compound 28p on motility of DU145 prostate cancer cells. DU145 cells were grown to confluence on laminin coated dishes, and induced to quiescence by serum starvation for 16 hr. DMSO vehicle control (A,B) or compound 28p (C,D) was added. After two hr, a scratch was made in the monolayer, using a sterile pipette tip. The cells were allowed to migrate into the scratch for 22 hr at 37^{o}C. A and C were photographed at 0 hr after scratch; B and D were photographed 22 hr after scratch. Compound 28p completely inhibited cell migration into the scratch.

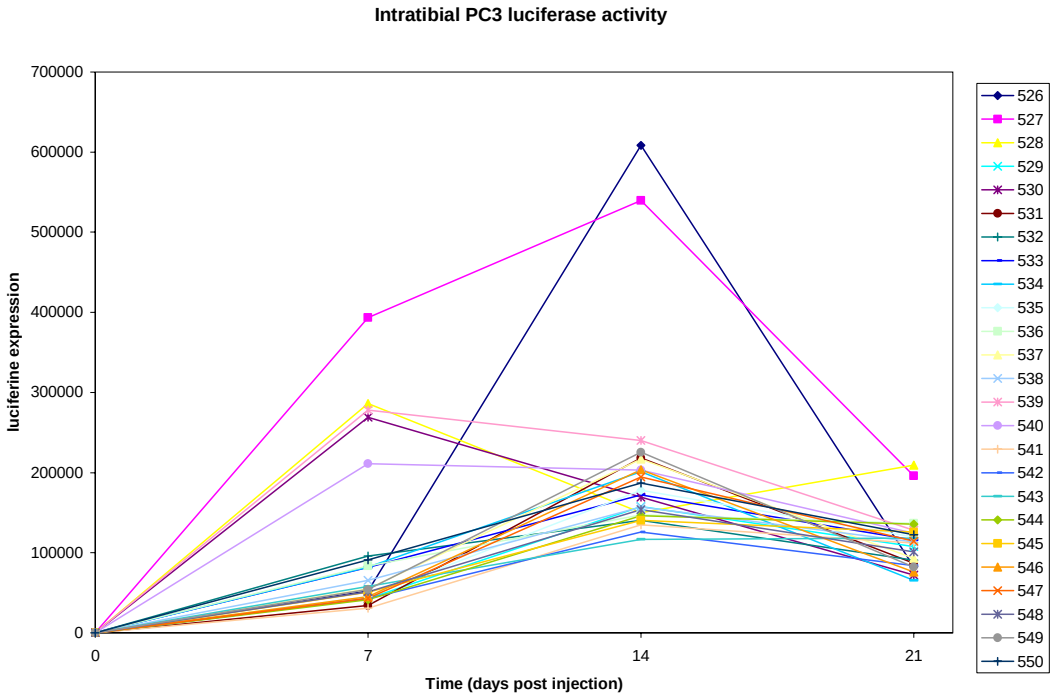

Fig. 2. PC3luc-vector only and PC3luc-CDK5dn cells were injected into the tibias of male nude mice. On day 0, 7, 14, and 21, mice were injected with D-luciferin and imaged on a Xenogen IVIS 100 bioluminescence imager. Since the threshold luciferase level for cell growth is 1 X 10^6 RLU, these data show that the tumor cells failed to grow. This was confirmed by direct examination of tibias after euthanasia of the mice.

Table 1. Sites examined for change in phosphorylation during CDK5 inhibition

Protein	phos. site	antibody	reference
PAK1	T212	Sigma PK-18	1,2
FAK	S732	Biosource 44-590	3
FAK	Y397[a]	Biosource 44-624G	3
Presenilin-1	T354	Cell Signaling 3622[b]	4
β-catenin	S191,S246	Transduction Labs C19220[b]	5
Ezrin	T235	Philip Hinds P235	6

[a] Not a direct substrate of CDK5

[b] Examined by immunoprecipitation, followed by Western blotting for pS/T (Cell Signaling 2321)

References for Table 1

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